[077] Double digestion of pEGF-C1 plasmid (Clontech, see figure) with K*pn*I and S*ma*I enzymes. After blunt ending the K*pn*I extension with "Mung bean" nuclease, the two extremities are ligated.

```
5'    GTC  GAC  GCT  ACC  GCG  GGC  CCG  GGA  TCC  3'3'
      CAG  CTG  CCA  TGG  CGC  CCG  GGC  CCT  AGG  5'
                 KpnI                   SmaI
```

(SEQ ID NO: 27)

```
                          ⇓

      GTC  GAC  GGT  AC                G    GGA  TCC
      CAG  CTG  C                       C    CCT  AGG

                          ⇓

      GTC  GAC  G                       G    GGA  TCC
      CAG  CTG  C                       C    CCT  AGG

                          ⇓

                GTC  GAC  GGG  GAT  CC
                CAG  CTG  CCC  CTA  GC
                     SalI             BamHI
```

(SEQ ID NO: 28)

Please replace paragraph 78 of the specification, which bridges pages 17 and 18, with the following paragraph:

Stage 2: pEGFP-CImut (GFP mutagenesis)

[078] Four mutagenesis oligonucleotides were used on a single-stranded molecule prepared using pEGFP-CIdKS. Each oligonucleotide comprises one or several mismatches (identified below in lower case letters), causing the desired mutation. In the pEGFP-CImut plasmid chosen, cut with the *Sac*II enzyme but not the *Age*I enzyme, all of the mutations were verified by sequencing.

-Destruction of the *Age*I site, introduction of a *Sac*II site and deletion of a Valine codon normally absent in "wild-type" GFP (Prasher, D.C., Eckenrode, R.K., Ward, W.W., Prendergast, F.G., and

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Cormier, M.J., Primary structure of the Aequorea victoria green-flourescent protein. Gene 111 (1992) 229-233.)

				Met		Ser	Lys	Gly	Glu
(SEQ ID NO: 30)									
		SacII							
oGM1:	5-'	GCGCTACCGcggGCCACC		ATG		AGC	AAG	GGC	GAG 3'
(SEQ ID NO: 29)									
pEGFP-C1dKS:	5'	GCGCTACCGGTCGCCACC		ATG	GTG	AGC	AAG	GGC	GAG 3'
		AgeI							
(SEQ ID NO: 31)					Val				

-Replacement of the 163 Valine codon by an Alanine codon in order to increase the quantity of GFP assuming a correct conformation at 37°C (Siemering, K.R., Golbik, R., Sever, R., and Haseloff, J., Mutations that suppress the thermosensitivity-of green flourescent protein. Current Biol. 6(1996) 1653-1663.)

			Ile	Lys	Ala	Asn	Phe	Lys	
(SEQ ID NO: 34)									
oGM2:	5'	GC	ATC	AAG	Gcc	AAC	TTC	AAG	3'
(SEQ ID NO: 33)									
pEGFP-C1dKS:	5'	GC	ATC	AAG	GTG	AAC	TTC	AAG	3'
(SEQ ID NO: 35)									
(SEQ ID NO: 36)				Val					

-Replacement of a 231 Leu codon by a Histidine codon normally present in "wild-type" GFP (Prasher, D.C., Eckenrode, V.K., Ward, W.W., Prendergast, F.G., and Cormier, M.J., Primary structure of the Aequorea victoria green-fluorescent protein. Gene 111 (1992) 229-233.)

		Ile	Thr	His	Gly	Met		
(SEQ ID NO: 38)								
oGM3:	5'	GG ATC	ACT	CaC	GGC	ATG	GA	3'
(SEQ ID NO: 37)								
pEGFP-C1dKS:	5'	GG ATC	ACT	CTC	GGC	ATG	GA	3'
(SEQ ID NO: 39)								

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```
                    Ile   Thr    His    Gly    Met
(SEQ ID NO: 38)
oGM3:         5' GG ATC   ACT    CaC    GGC    ATG GA 3'
(SEQ ID NO: 37)
pEGFP-CIdKS: 5' GG ATC   ACT    CTC    GGC    ATG GA 3'
(SEQ ID NO: 39)
(SEQ ID NO: 40)                 Leu
```

Please replace paragraph 79 of the specification, on pages 18-20, with the following paragraph:

[079] Four PCRs (Polymerase Chain Reaction) done on a vector comprising the aequorin (Aeq) coding phase makes it possible to amplify the A, B, C, and D fragments with, respectively, the primers oAE5A and oAE3A, oAE5B and oAE3B, oAE5C and oAE3C, oAE5D and oAE3D. The overlapping regions are used to assemble the different parts during successive PCRs (Ho, S.N., Hunt, H.D., Horton, R.M., Pullen, J.K., and Pease, L.R. Site-directed mutagenesis by overlap extension using the polymerase chain reaction Gene 77 (1989) 51-49.) An A+B fragment is amplified starting with a mixture of A and B fragments, and primers oAE5A and oAE3B. Similarly, a C+D fragment is amplified with a mixture of C and D fragments, using the primers oAESA and oAE3D. Finally, the complete coding phase, A+B+C+D is developed with the primers oAE5A and oAE3D.

- Each oligonucleotide comprises one or several mismatches that are identified below in lower case. The "wild" sequence is represented opposite, in upper case. The primer oAE5A suppresses the original initiation translation code (ATG) and introduces a B*g*lII site. The primer oAE3D introduces an X*ho*I site just behind the translation

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terminal codon (TAA). The final PCR product, digested with the B*g*lII and X*ho*I enzymes, is cloned in the B*g*lTI-S*a*lI sites of the pEGFP-Clmut plasmid in such a way that the Valine codon (GTC), the first codon of aequorin, is the same reading phase as the GFP (see figure). The other primers introduce "silent" mutations that do not change the protein sequence but modify six codons in the jellyfish, *Aequoria Victoria*, to improve their expression in mammals (Wada, K-N., Aota, S.-I., Tshuchiya, R., Ishibashi, F., Gojobori, T., and Ikemura, T. Codon usage tabulated from the GenBank genetic sequence data. Nucleic Acids Res. 18 suppl. (1990) 2367-2411). The completeness of the entire sequence was verified by sequencing,

```
        oAE5A    CCATG
5'      AGCTTCAgatct GTC AAA CTT ACA TCA GAC TTC GAC AAC CCA AGA TGG ATT GGA CGA
3'      TCGAAGTctaca CAG TTT GAA TGT AGT CTG AAG CTG TTG GGT TCT ACC TAA CCT GCT
            BGlII
CAC AAG CAT ATG TTC AAT TTC CTT GAT GTC AAC CAC AAT GGA AAA ATC TCT CTT GAC GAG
GTG TTC GTA TAC AAG TTA AAG GAA CTA CAG TTG GTG TTA CCT TTT TAG AGA GAA CTG CTC
ATG GTC TAC AAG GCA TCT GAT ATT GTC ATC AAT AAC CTT GGA GCA ACA CCT GAG CAA GCC
TAC CAG ATG TTC CGT AGA CTA TAA CAG TAG TTA TTG GAA CCT CGT TGT GGA CTC GTT CGG
                oAE5B  A
AAA CGA CAC AAA GAT GCT GTg GAA GCC TTC TTC GGA GGA GCT GGA ATG AAA TAT GGT GTG
TTT GCT GTG  TTT CTA CGA CAc CTT CGG AAG AAG CCT CCT CGA CCT TAC TTT ATA CCA CAC
                              T         oAE3A
GAA ACT GAT TGG CCT GCA TAT ATT GAA GGA TGG AAA AAA TTG GCT ACT GAT GAA TTG GAG
CTT TGA CTA ACC GGA CGT ATA TAA CTT CCT ACC TTT TTT AAC CGA TGA CTA CTT AAC CTC
                        oAE5C  G      T   A
AAA TAC GCC AAA AAC GAA CCA ACc CTC ATC CGc ATc TGG GGT GAT GCT TTG TTT GAT ATC
```

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```
TTT ATG CGG TTT TTG CTT GGT TGg GAG TAG GCg TAg ACC CCA CTA CGA AAC AAA CTA TAG

                                C       A   T oAE3B

GTT GAC AAA GAT CAA AAT GGA GCT ATT ACA CTG GAT GAA TGG AAA GCA TAC ACC AAA GCT

CAA CTG TTT CTA GTT TTA CCT CGA TAA TGT GAC CTA CTT ACC TTT CGT ATG TGG TTT CGA


GCT GGT ATC ATC CAA TCA TCA GAA GAT TGC GAG GAA ACA TTC AGA GTG TGC GAT ATT GAT

CGA CCA TAG TAG GTT AGT AGT CTT CTA ACG CTC CTT TGT AAG TCT CAC ACG CTA TAA CTA

                                          oAE5D  A      T A

GAA AGT GGA CAA CTC GAT GTT GAT GAG ATG ACA AGA CAg CAT cTg GGA TTT TGG TAC ACC

CTT TCA CCT GTT GAG CTA CAA CTA CTC TAC TGT TCT GTc GTA gAc CCT AAA ACC ATG TGG

                                                  T     A T           oAE3C

                                                                XhoI

ATG GAT CCT GCT TGC GAA AAG CTC TAC GGT GGA GCT GTC CCC TAA TCTcGAGGATCTTT 3'

TAC CTA GGA CGA ACG CTT TTC GAG ATG CCA CCT CGA CAG GGG ATT AGAgCTCCTAGAAA 5'

                                                              T     oAE3D
```

(SEQ ID NO: 41)

Please replace paragraph 80, bridging pages 20 and 21 of the specification, with the following paragraph:

[080] In the pEGFPmur-Aeq plasmid, a sequence of five amino acids exists between the coding phases of the GFP and aequorin. Observations led to the lengthening of this region by intercalating a sequence in the B*sp*EL site. Two complementary oligonucleotides coding for a sequence of nine amino acids give the composition a good deal of flexibility, owing to the abundance of Glycine and Serine. After insertion, the B*sp*EI site is preserved on only one side although new intercalated sequences may be added successively. At each stage, the orientation is controlled by

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the B*sp*EI enzyme. Two copies of this sequence are needed to restore the normal fluorescence of GFP, but the energy transfer between aequorin and GFP is optimal with five copies. The entire intercalated sequence of pGCA plasmid (5 x 9 aa + the five initial amino acids =50 aa) was verified by sequencing:

```
        Lys   Ser   Gly   Leu   Arg   Ser   Val          (SEQ ID NO: 43)
5'      AAG   TCC   GGA   CTC   AGA   TCT   GTC   3'     (SEQ ID NO: 42)
3'      TTC   AGG   CCT   GAG   TCT   AGA   CAG   5'     (SEQ ID NO: 44)

        GFP   BspEI       BGlII Aeq

                    ⇓

5'      AAG   T     GC    GGA   CTC   AGA   TCT   GTC   3'(SEQ ID NO: 45)
3'      TTC   AGG    CC     T    GAG   TCT   AGA   CAG   5'(SEQ ID NO: 44)

                                +

        Gly   Gly   Ser   Gly   Ser   Gly   Gly   Gln   Ser   (SEQ ID NO: 47)
5'CC    GGC   GGG   AGC   GGA   TCC   GGC   GGC   CAG   T      3'  (SEQ ID NO: 46)
3'___G  CCC   TCG   CCT   AGG   CCG   CCG   GTC   AGG   CC 5'(SEQ ID NO: 48)
                  BamHI                     BspEI
```

IN THE CLAIMS:

Please cancel claims 4-8, 10-14, 20-25, 28-31, and 34-37

Please amend the claims as follows:

1. (Amended) A method of screening *in vivo* for a change in a physical, chemical, biochemical, or biological, condition, the method comprising the steps of:

a) administering to a mammal a composition comprising a bioluminescent system;

b) detecting whether light is produced; and

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